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Lane E. Breshears ,   Brandon T. Nguyen ,   Samantha Mata Robles ,
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Biosensor detection of airborne respiratory viruses such as SARS-CoV-2

Lane E. Breshears[†], Brandon T. Nguyen[†], Samantha Mata Robles, Lillian Wu, and Jeong-Yeol Yoon*
Department of Biomedical Engineering, The University of Arizona, Tucson, Arizona 85721, United State

[†]These authors contributed equally.
*Corresponding author. E-mail: jyyoon@arizona.edu

Abstract. Airborne SARS-CoV-2 transmission represents a significant route for possible human infection that is not yet fully understood. Viruses in droplets and aerosols are difficult to detect because they are typically present in low amounts. In addition, the current techniques used, such as RT-PCR and virus culturing, require large amounts of time to get results. Biosensor technology can provide rapid, handheld, and point-of-care systems that can identify virus presence quickly and accurately. This paper reviews the background of airborne virus transmission and the characteristics of SARS-CoV-2, its relative risk for transmission even at distances greater than the currently suggested 6 feet (or 2 m) physical distancing. Publications on biosensor technology that may be applied to the detection of airborne SARS-CoV-2 and other respiratory viruses are also summarized. Based on the current research we believe that there is a pressing need for continued research into handheld and rapid methods for sensitive collection and detection of airborne viruses. We propose a paper-based microfluidic chip and immunofluorescence assay as one method that could be investigated as a low-cost and portable option.

Keywords: COVID-19, aerosol, droplet, air sampling, point-of-care.

Background

The world is facing a new public health crisis in the emergence and spread of the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus likely originated in bats and was transmitted to humans through a still unknown intermediary vehicle[1]. Research continues to reveal that in addition to transmission through droplets, like many other respiratory viruses, inhalation of fine aerosols (< 5 μm in diameter) with sufficient viral load can lead to infection of susceptible individuals. Many individuals infected with SARS-CoV-2 are asymptomatic, and studies have shown that there can be no difference in viral burden between symptomatic and asymptomatic patients[2].

Since 2015 and the publication of our last review on biosensors for monitoring airborne pathogens[3], there have been multiple advances in developing biosensors for detection of respiratory viruses. The COVID-19 pandemic poses a new challenge due to its high rate of contagion, mortality, and impact on not only healthcare systems, but also economics and policy. Great strides have been made to rapidly develop new tests and biosensors for detecting SARS-CoV-2 in complex mediums like saliva and nasal secretions; however, there are few available tests or products for detecting airborne SARS-CoV-2 (we found one portable PCR product advertised for airborne SARS-CoV-2 detection[4]). Such a biosensor would help tremendously in both clinical settings and public settings like restaurants and stores. Research has revealed that early protocols for the physical distancing of 6 feet (or 2 m) may not be sufficient for preventing the airborne spread of SARS-CoV-2[5–7]. Airborne respiratory virus biosensors could lead to a paradigm shift in both early detection and prevention of worldwide pandemics. As discussed in our review article published in 2015 on airborne detection, biosensors have been traditionally used to detect specific or nonspecific biological analytes either directly or indirectly using a variety of evolving methods[3]. Biosensors continue to improve, becoming portable, specific, sensitive, and easy to use. The limiting factor for airborne virus biosensors continues to be the low concentration of the target virus in the environmental sample. This review focuses on collecting recent research published since 2015 about biosensors for airborne pathogen detection. Our objective for this review is to provide a resource for developing biosensors for detecting respiratory viruses, such as SARS-CoV-2.

New species of human viruses are appearing at a rate of three or four per year[8]. Novel strains of the SARS-CoV-2 continue to be identified with origins around the world with varying degrees of infectivity and severity of infection[9]. These novel strains arose due to the large number of cases worldwide, therefore early detection of a novel respiratory virus is paramount to preventing pandemics. As mentioned earlier, few efforts have been successful in detecting airborne respiratory viruses like SARS-CoV-2 from environmental samples. Here we examine the state of biosensors for airborne respiratory viruses with case-studies and examination of current laboratory-based methods.

Aerosols, Droplets, and Collection/Detection Methods

Aerosols and Droplets

The World Health Organization (WHO) defines airborne transmission as, "the spread of an infectious agent caused by the dissemination of droplet nuclei (aerosols evaporated from larger droplets) that remain infectious when suspended in air over long distances and time"[10] (Figure 1). Respiratory secretions can be aerosolized through daily activities like exhaling, talking, coughing, and sneezing, as well as medical procedures including tracheal intubation, bronchoscopy, and tracheotomy[11,12]. Medical procedures that produce aerosols place healthcare

providers at heightened risk when treating patients that may be infected with COVID-19. These procedures include endonasal and transsphenoidal procedures. The scientific community has been discussing the possibility of SARS-CoV-2 spread through aerosols outside of aerosol generating procedures. Current theories and studies of physics surrounding exhalation have generated hypotheses that 1) respiratory droplets generate microscopic aerosols less than 5 µm in diameter, and 2) normal breathing and talking results in the generation of aerosols[13]. Infection via aerosol inhalation would require a sufficient quantity of virus present in the aerosol, however, the proportion of exhaled droplet nuclei that generate aerosols and the infectious dose required to cause infection in another person are not known.
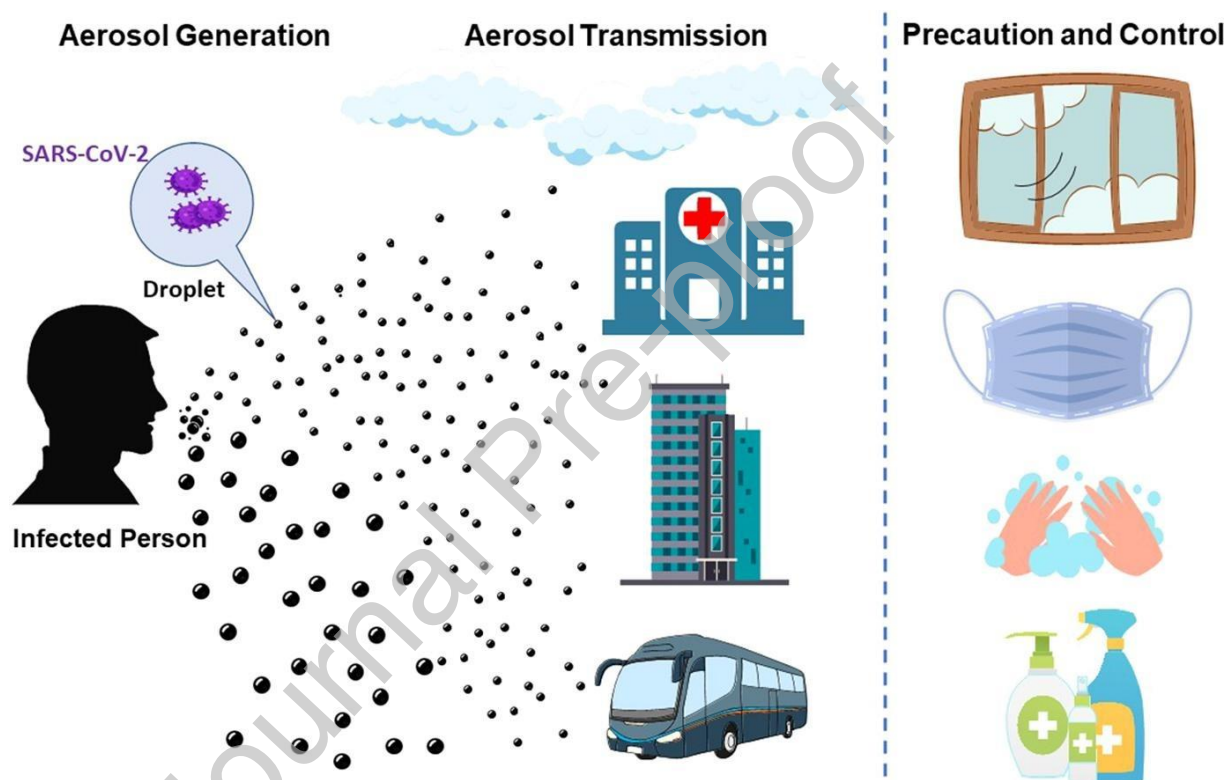
Figure 1. Aerosol/droplet generation, their transmission, and the precaution/control measures. Reprinted from ref. 11 with CC BY license, (c) 2020 Tang et al.

After initial aerosolization, virus particles can deposit on surfaces and aggregate into larger droplets. The virus can remain viable on surfaces for days in favorable atmospheric conditions but are destroyed in less than a minute by common disinfectants like sodium hypochlorite, hydrogen peroxide, etc.[14,15]. This deposited material can be re-aerosolized by human activities (e.g., walking, cleaning, removing PPE, and door opening)[11,16]. In clinical reports of healthcare workers exposed to COVID-19 cases in the absence of aerosol-generating procedures, there were no nosocomial infections, also known as hospital acquired infections (HAIs), found when contact and droplet precautions were properly employed. These precautions

included the wearing of medical masks as a component of PPE (personal protective equipment) (Figure 1).

Typical airborne SARS-CoV-2 concentrations have been measured in different hospital locations ranging from 1 to 42 copies m^{-3} depending on the location and conditions of sampling[17]. Liu et al. found that the highest range of airborne viruses was from restrooms with toilets and the Protective Apparel Removal Rooms; however, this was then reduced to negative test results with increased sanitization processes and reduced medical staff[17]. Contrary to the initial speculation that aerosol transmission of SARS-CoV-2 is unlikely, further studies have detected airborne samples, as discussed in the next section. Outside of medical settings, there have been reports of outbreaks in crowded indoor spaces, where sufficient physical distancing and lack of ventilation over a prolonged period could exacerbate aerosol and droplet transmission. Table 1 summarizes the possible transmission routes of respiratory viruses.

Table 1. Characteristics of airborne particles.

Transmission mode	Diameter[18]	Travel time[19]	Travel distance[18]
Droplet	> 5 µm	Varies depending on ambient conditions	Up to 1 m
Droplet nuclei or aerosol	< 5 µm	Dozens of minutes - Hours	> 1 m
Contact or surface transmission	Varies	N/A	N/A

The detection of airborne viruses is largely like the processes used for non-bioaerosol sampling. This is thoroughly discussed by Hinds[20], and the three key stages for bioaerosol sampling are described and compared to those used for non-bioaerosols (Figure 2). Air sampling is affected by the condition of the indoor environment and the placement of the sampler. Depending on any air-conditioning present, and the level of ventilation, the viability of the virus and the movement it takes throughout the room will vary. Based on these factors, it is important to place the air sampler in an area that will receive the highest probability of interacting with the bioaerosols. After air sampling, the droplets or aerosols are collected through impaction or deposition onto a chosen media for later detection of target microorganism and/or evaluation of concentration.
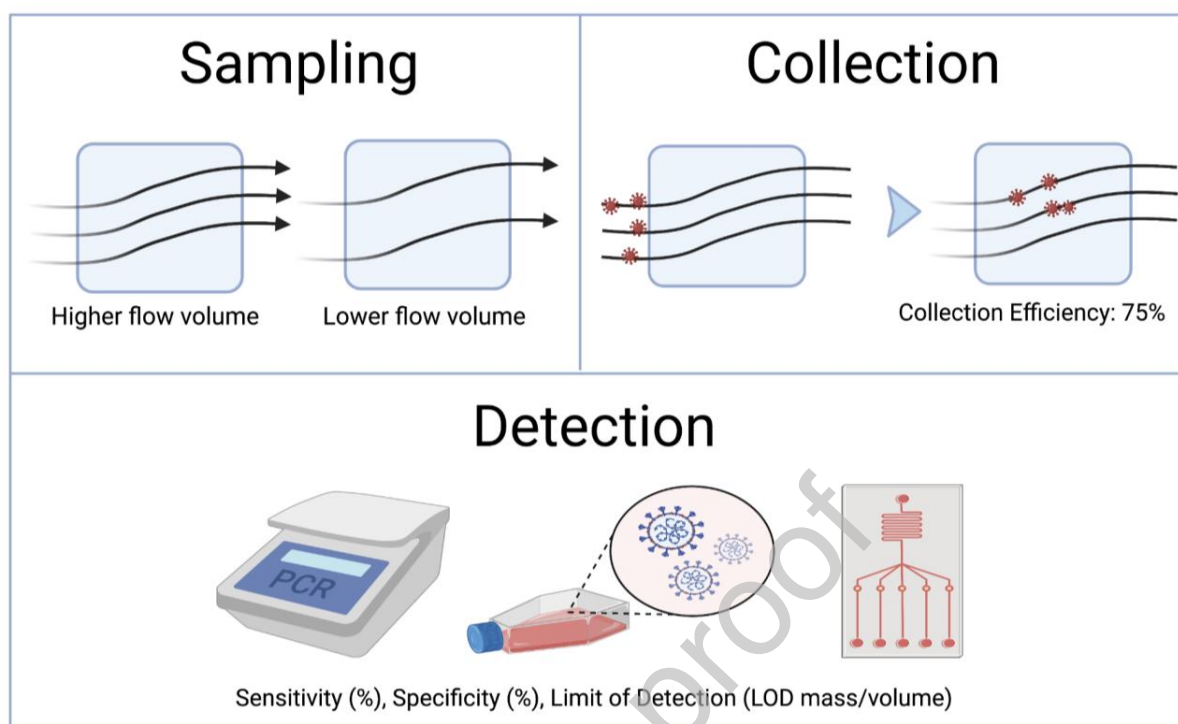
Figure 2. The typical process for bioaerosol detection goes in three steps. (1) Sampling, which is measured in flow volume (L/min). (2) Collection, which is measured by collection efficiency. (3) Detection method, including (left to right) RT-PCR, virus culturing, microfluidics, and more. Detection method successfulness is based on the sensitivity, specificity, and limit of detection (LOD). Schematic was made using BioRender.com.

Sample Collection Techniques

Slit impactors impact particles directly onto a medium[20]. For virus particles, a cell or tissue culture media would be used[20]. Many impactors also use cutoff diameters to specify certain particle sizes. A cutoff size of 2 μm would result in 50% of particles collected being 2 μm in size[20]. Certain techniques are designed to prevent overloading of particles in single areas. Overloading makes identification of target particles difficult and typically leads to underestimation or nonspecific identification. Due to the shear forces caused by high-flow samplers, virus viability may also become an issue during the impaction process[20,21]. Low-flow samplers can be used to maintain virus viability, but will decrease the collection efficiency. Other types of impactors worth noting are those used to collect pollen, where culture is not an option. One type, the Rotorod sampler[20,22], directly impacts pollen onto adhesive-coated polystyrene-rods. This could be an interesting alternative to explore for virus collection, as we did not find any articles taking this particular approach.

Impingers are similar to impactors except the jet portion is submerged in a liquid, typically water or alcohol[20]. Liquid collection prevents any desiccation of airborne virus samples, however again the shear forces in the turbulent liquid can result in loss of viability. Depending

on the cutoff level of the impinger, the loss of viability can be decreased (lower cutoff = larger loss of viability). Typically, the water from the impingers is then used for culturing.

A centrifugal sampler uses rotation to pull particles in[20]. They typically achieve sample flow rates of 40 to 50 L/min. Respirable sampling and inhalable particle sampling are especially useful for larger particles, which can become platforms for viral particle transmission[23].

Detection Techniques

The third stage, microorganism detection from the collected sample, is the key difference between bio- and non-bioaerosol detection. This is also one of the key components required for accurate SARS-CoV-2 detection. After particle collection, there needs to be (a) confirmation of specificity (i.e., whether we measure our target particle or not), and (b) quantification of the virus. For binary assays, it is possible that you would not need to determine exact amounts of virus. Some of the collection methods assume that the virus will be grown on a cell culture medium which requires time and attention to achieve accurate results. Culturing is also difficult because it requires viable virus samples, which is often difficult to achieve with the high-flow samplers. In addition, there are possibilities of underestimation or non-specificity if oversampling has occurred[20]. Some viruses are not easily cultured, especially from low concentrations that you would find from bioaerosol samples.

Membrane filters, also potentially including microfluidic platforms, are often used for bioaerosol collection in highly contaminated environments[20,24,25]. The particles can be directly examined from the filter using an optical microscope or can be cultured by placing the filter on a culture medium. Although there are no intense shear forces present like in the previous methods, the filtration method does still cause significant desiccation and loss of viability.

Nucleic acid amplification methods, such as real-time quantitative polymerase chain reaction (RT-qPCR) or loop-mediated isothermal amplification (LAMP), are often the chosen technique for virus detection due to high sensitivity, specificity, and ability to amplify small concentrations of the sample[3,26,27]. These methods are useful for airborne virus detection because they can handle low concentrations. Despite the many advantages of nucleic acid detection methods, these require multiple hours, expensive equipment, and extensive training to perform. An alternative approach is antigen-antibody binding assay, such as lateral flow assay (LFA) and sandwich immunoassay, which uses prepared antibodies and reporter probes (gold nanoparticles, fluorescent particles, etc.)[3]. These methods are typically low-cost, rapid, and much easier to perform. Like nucleic acid amplification, this method does not require viable virus particles for accurate detection. Commercial kits with point-of-care diagnostic capabilities will often use immunoassays for on-site results. Some disadvantages to immunoassays are false positives, the hook effect, and difficulty maintaining specificity when using complex samples. Finally, some other methods, such as surface-enhanced Raman spectroscopy (SERS) or surface plasmon resonance (SPR), can detect target virus particles using optical properties, often enhanced by using antibodies[3].

Respiratory Viruses

Influenza viruses are among the most common and infectious respiratory viruses worldwide and are spread easily through coughing, sneezing, and even quiet breathing, as they form both large droplets and small aerosols[28]. SARS-CoV-2 has proven to be one of the deadliest and contagious respiratory viruses to affect the world recently. Contracting COVID-19 can cause symptoms including but not limited to fever, cough, sore throat, loss of smell, headache, and body aches[1]. Severe infection and fatalities occur more frequently in individuals with underlying medical conditions, called co-morbidities.

Viral respiratory infections arise when a virus infects the cells of the respiratory mucosa via exposure through inhalation of virus particles or direct contact with the mucosal surface of the nose or eyes (Figure 3)[2,28].
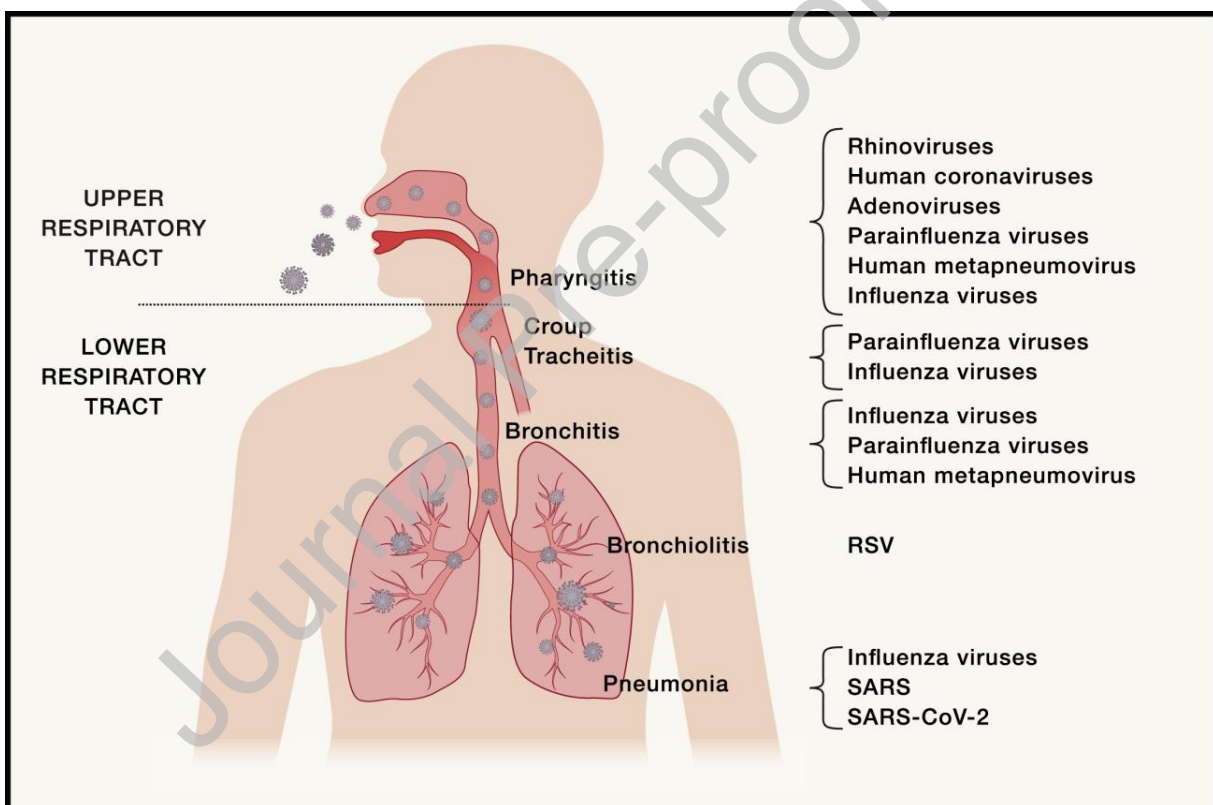
Figure 3. Schematic illustration of the human respiratory tract, indicating the clinical presentations associated with different respiratory viruses that infect the parts of the upper and lower respiratory tracts. Reprinted from Subbarao et al. ref. 28 with permission, (c) 2020 Elsevier.

Viruses take advantage of the existing machinery of cells to replicate. The basic structure of a virus is composed of genome (DNA or RNA), protein capsid, and in some cases a lipid envelope covering the capsid[29]. In viral respiratory infections, the human body possesses

physical barriers in the form of epithelial cells and mucus alongside alveolar macrophages in the lungs[29]. These barriers aid and supplement the immune response of the host body. Despite these protections, viruses can mutate and become more infectious by evading the immune system and aggravating the clinical condition of the host. SARS-CoV-2 and other respiratory viruses like severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), respiratory syncytial virus (RSV), and influenza A/H1N1 (Table 2) are RNA based, leading to a higher rate of mutation[30]. Both SARS-CoV and SARS-CoV-2 have similar core sizes of about 100 nm and an average spike size of 23 nm[31]. The lower fidelity of RNA polymerase results in more nucleotide errors during the replication of the virus genome.

Table 2. Respiratory viruses and their dimensions.

Virus	Diameter
SARS-CoV-1	82-94 nm[32]
SARS-CoV-2	60-140 nm[33–35]
Murine hepatitis virus (MHV)	85 nm[36]
MERS-CoV	100 nm[37]
Influenza A/H1N1	80-120 nm (0.08-0.12 μm)[38]
RSV	150-250 nm (0.15-0.25 μm)[39,40]

SARS-CoV-2 Airborne Transmission

SARS-CoV-2 is an enveloped virus with 0.1 μm in diameter (Table 2) and can be transmitted in larger droplets (> 5 μm) and smaller ones (< 5 μm) (Table 1). These smaller droplets are aerosols, including droplet nuclei that are evaporated from larger droplets. Transmission of some viruses, like measles, has been demonstrated via aerosols[21]. In a modelling report on the physics of SARS-CoV-2 transmission via aerosol and droplet dispersion, airborne transmission is defined as any pathogen that can be transmitted via air as: aerosols, droplets, or dust[41]. Any droplet with a diameter greater than 0.1 μm (typical diameter of viruses; Table 2) suspended in the air that is inhaled by a susceptible individual has the potential to carry a viral vector and result in infection. Despite this potential risk, Lee estimated that the minimum size of respiratory particle required to maintain the mass of a SARS-CoV-2 virus particle depends on the percentage of respiratory fluid. For example the absolute minimum size would be 0.09 μm, corresponding to the size of a single virion, but if only 10% of the respiratory fluid is occupied by SARS-CoV-2 then the minimum respiratory particle size would be 42 μm[23]. This suggests that only the largest aerosol particles may pose a risk for virus transmission. However, Liu et al. measured airborne SARS-CoV-2 in Wuhan hospitals and found that peak airborne SARS-CoV-2

concentrations were measured in the count of 40 and 9 copies m^{-3} in 0.25-0.5 μm and 0.5-1.0 μm ranges respectively, and at 7-9 copies m^{-3} in the supermicron size distribution[17].

Presently, airborne transmission is not well understood for any virus, including SARS-CoV-2. There is little known about the relationship between aerosol particle size range versus the number of infectious viruses within a given particle. Evidence for airborne transmission of SARS-CoV-2 continues to be reported; however, many cases lack confirming studies and supplemental data. Studies surrounding the size distribution of SARS-CoV-2 droplets generated by breathing, speaking, coughing, and sneezing report varying results, with particle sampling being the main contributor to variation.

The main gaps in research surrounding airborne transmission of SARS-CoV-2 include: (1) the role of coughing, speaking, and breathing in the formation of aerosols and droplets, (2) travel distance and time of airborne particles, and (3) risk analysis for common indoor scenarios like restaurants, apartments, etc. and the contribution of ventilation and air conditioning to airborne spread.

Chia et al. reported SARS-CoV-2 RNA in aerosol particles of 1-4 μm and > 4 μm from air samples collected from two different ICU isolation rooms in Singapore[42]. Another case study from Seoul, South Korea found evidence pointing strongly at aerosol transmission of COVID-19 as the main contributor to an isolated outbreak in an apartment building[43]. The infections in the apartment were found along two vertical lines (Figure 4) in the building, wherein the rooms along each vertical line were connected via a natural ventilation shaft. In total, there were 10 positive cases of COVID-19 in the building out of 437 residents from 267 households. 8 of the positive cases were connected via units along the same vertical shaft, and the remaining two were connected via a separate vertical shaft. Investigation into the backgrounds of infected residents yielded no relationships or history of interpersonal contact. Droplet infection via surfaces like elevators and railings is still possible, but from this work it is reasonable to consider aerosol transmission through the ventilation shafts as a potential route. This study, like others, is limited by the lack of sample collection and confirmation of airborne SARS-CoV-2.

A recent study found that SARS-CoV-2 can remain infective in aerosols < 5 μm for 3 hours and 72 hours on surfaces under laboratory conditions[41]. In addition, several studies referenced by the WHO[13] found that experimentally created airborne SARS-CoV-2 could remain within aerosols from up to 3 hours[44] or 16 hours[44].

A. Confirmed Households

B. Characteristic of Unit Plan
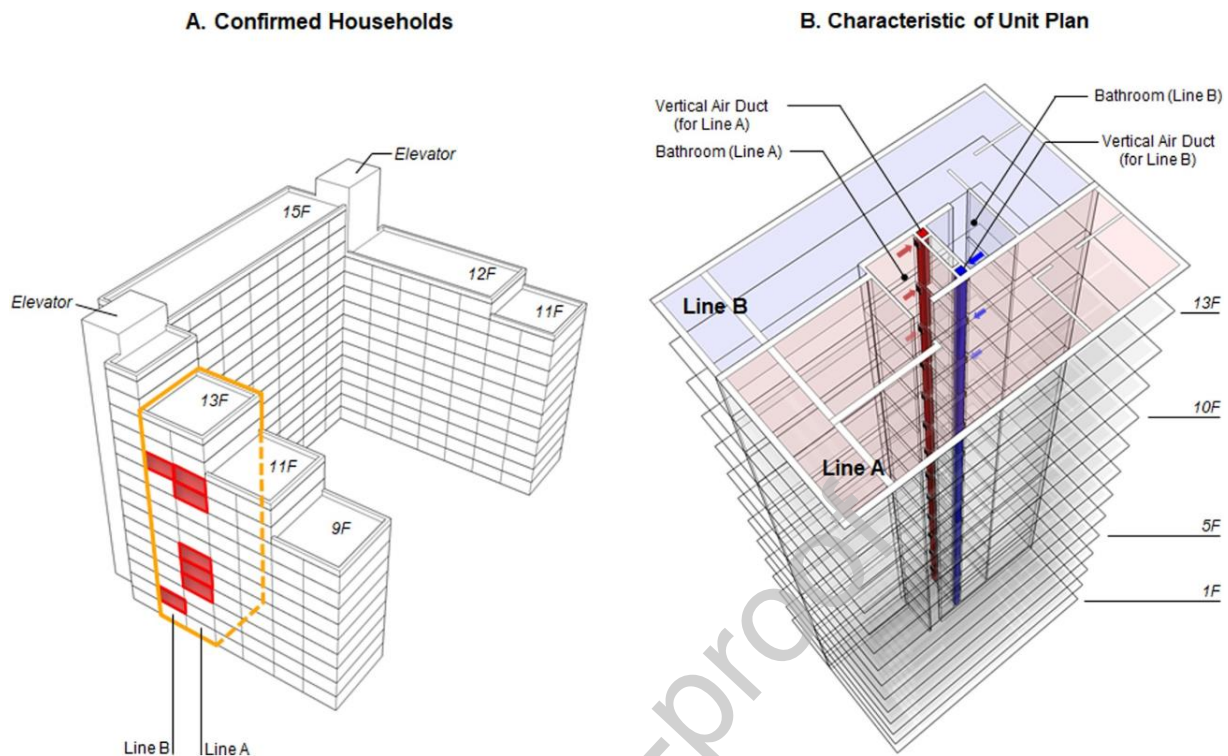
Figure 4. Structure of the apartment building where the infection outbreaks occurred in two vertical lines. (A) Confirmed units. (B) Characteristics of the unit plan. Reprinted from Hwang et al. ref. 43 with permission, (c) 2021 Elsevier.

A recent study found that in short-range contact (< 2 m or 6 ft) that airborne transmission route dominates at most distances compared to large droplet transmission[45,46]. The airborne transmission route typically refers to the aerosols (< 5 μm) that are inhaled, leading to infection. Because this transmission route dominates at most distances and appears to travel farther than large droplets, there are significant implications about acceptable physical distancing in indoor settings. If it is shown that a viable SARS-CoV-2 virus can be carried in sufficient loads via aerosols, the 2 m (or 6 ft) distancing may not be effective in preventing the spread of the virus[5]. Additionally, PPE that does not filter out smaller particles may prove ineffective in protecting against airborne transmission when physical distancing cannot be maintained, such as in hospital settings and during medical procedures.

Table 3 summarizes publications performing airborne SARS-CoV-2 particle detection and subsequent sample collection and detection methods. Google Scholar was used as the database, with the keywords "airborne SARS-CoV-2 detection". The first 100 papers were recorded. Review articles, papers published before 2020, pre-prints, and papers not discussing airborne coronavirus were immediately excluded. The methods section of these papers was assessed to record sample collection and detection methods.

Table 3. Survey of (a) sample collection, and (b) detection techniques used in recent (2020-2021) articles detecting coronavirus from airborne samples. Bold references will also be discussed in the next section on biosensors for bioaerosol detection.

(a) Sample collection techniques				
Technique		**Advantages**	**Disadvantages**	**References**
Collected onto solid	Impactor, pump	Wide flow-rate range (10-1200 L/min) Fast collection time	Shear forces damage particle viability Lower collection efficiency	Liu et al.[17], Razzini et al.[46], Stern et al.[47], Moreno et al.[48], Chirizzi et al.[49], Rodriguez et al.[50], Dumont-Leblond et al.[51], Jin et al.[52], Barbieri et al.[27], Stern et al.[53]
	Centrifugal sampler	Higher collection efficiency Higher flow volume	High shear forces	Schuit et al.[54]
	Cyclone	Higher collection efficiency Higher flow volume	High shear forces	Chia et al.[42], Hirota[55]
	Electrostatic	Low-flow rate Increased particle attraction No shear force affecting particle viability High collection efficiency	Charge may damage particle viability Slow collection time	**Kim et al.[56], Piri et al.[57]**
	Swabbing	Does not require aerosol sampling Convenient Low-cost May have higher concentration than in aerosol format	Does not accurately represent airborne concentration Deposited samples may no longer be viable	Moreno et al.[48], Rodriguez et al.[50], Nissen et al.[58], Moitra et al.[59], Maestre et al.[60]
	Passive sampling (no induced air sampling)	Simple setup (no electricity, battery, etc.) Portable Typically used with culturing	No air flow = lowest collection efficiency Placement of sampler highly influences results	Baboli et al.[61]

		methods		
Collected into liquid	Impinger	Liquid collection	Shear forces damage particle viability	Zhang et al.[59], Baboli et al.[61], Zhou et al.[62], Kenarkoohi et al.[63], Faridi et al.[64]
	ATH enrichment	Fast sampling time (1 min) Volume reduction enrichment Combined enrichment and collection method	Requires specific liquid	**Kim et al.[56], Piri et al.[57],** Hu et al.[65]
	Microfluidics	Able to mix samples Portable Low-cost Able to modify Good for ATH and HTH enrichment Maintain particle viability	Very low air flow rates (<1 L/min) may result in low collection efficiency Requires pipetting and transfer of samples into liquid form	**Xiong et al.[66]**

(b) Detection techniques

Technique		Advantage	Disadvantage	References
Intact virus required	Culturing	Simple technique Provides information on whole aerosol sample	Oversampling can make plaque counting difficult Contamination of samples Time-consuming Requires viable viruses	**Kim et al.[56],** Nissen et al.[58]
Nucleic acid amplification	PCR	Highly sensitive Highly specific Can handle complex samples Detects low concentrations	Expensive Requires training to perform	Liu et al.[17], Barbieri et al.[27], Chia et al.[42], Razzini et al.[46], Stern et al.[47], Moreno et al.[48], Chirizzi et al.[49], Rodríguez et al.[50], Dumont-Leblond et al.[51], Jin et al.[52], Stern et al.[53], **Kim et al.[56], Piri et al.[57],** Nissen et al.[58], Maestre et al.[60],

				Baboli et al.[61], Zhou et al.[62], Kenarkoohi et al.[63], Faridi et al.[64], Hu et al.[65], **Xiong et al.**[66], Zhang et al.[67], Song et al.[68], Lednicky et al.[69], Lednicky et al.[70]
	LAMP	Highly sensitive Highly specific Single temperature requirement only Detects low concentrations	Expensive Requires training to perform	**Rahmani et al.**[71]
Antigen-antibody	ELISA and immunoassay	Highly sensitive Highly specific Detects low concentrations Multi-target detection Typically low-cost Optical signal	May require training to perform Requires preparation of antibody solution False-positives	**Piri et al.**[57], Moitra et al.[59]

Articles listed in bold used specifically biosensors for either sampling and/or detection of SARS-CoV-2 or HCoV-229E, and these will be discussed in further detail later in this review. This collection of airborne coronavirus detection clearly shows a trend for using impactor air samplers combined with nucleic acid amplification detection. PCR techniques were almost used by all articles published on detecting airborne SARS-CoV-2, proving it is the industry standard for accurate viral quantification. This dominance is likely due to the commercial availability of impactor-style air samplers; however, justification for the choice of air sampler was not typically discussed and is therefore difficult to assess. Impingers were the second most common air sampler, also often commercial products. Many impactors also used additional filtration membranes for the collection of particles after sampling. Gelatine[17,46,50,51] and glass fiber[27,47,53] were most commonly used for such filters. Immunoassays, although common for biosensor-type diagnostics, as seen in Table 4, were not typically used by articles assessing coronavirus presence from airborne samples.

Some articles opted to estimate airborne concentration by taking surface swabs at key sites for airflow, such as ventilation filters, to infer the number of airborne particles based on the concentration detection on the surface via deposition[58]. This technique may be helpful for easy estimation of the target. Still, it can overestimate airborne virus if concentration has occurred or underestimate if airflow does not permit high amounts of deposition in the area swabbed. In addition, this technique is unique because it does not directly sample airborne particles. The rest of the methods discussed directly sampled airborne particles.

Recent Biosensor Advances for Airborne Respiratory Viruses

Literature Survey

While many publications have been published reporting coronavirus detection from airborne samples (see Table 3), only a small number (marked bold) focused on the development of biosensor devices for this detection. Biosensors provide a low-cost and portable alternative to standard air sampling and detection methods; however, more research is needed on novel and improved methods for airborne detection of respiratory viruses. In addition, the field has made considerable advances in the realm of liquid media, such as water and saliva. Still, there remains a gap in airborne particle sampling, collection, and detection.

This review assessed recently published articles that were either directly related to biosensors for aerosol detection or were closely related to the sampling, collection, or detection steps. Using Google Scholar, we searched for publications using the keywords "biosensors," "airborne," "aerosol," and "virus." As some papers may not use the terminology biosensors, we then added the keywords, "point-of-care," "microfluidic," and "lab-on-a-chip." We then filtered all results from these searches to exclude any papers published before 2015. We also performed an additional search to include advances in detection methods with the potential to detect low

concentrations of viral particles. For this extra step, we did a Google Scholar search for publications using the keywords, "biosensors," "SARS-CoV-2," and "detection." We then used manual revision of the remaining papers to eliminate the papers that did not specifically discuss airborne detection or the development of sampling, collection, or detection modality for airborne detection. All papers were then categorized based on the target pathogen tested with the device, prioritizing respiratory viruses, including coronaviruses and influenza viruses. Due to the low number of publications on respiratory viruses, we also included the papers focusing on bacteria or polystyrene particles (surrogates for bacteria). However, they are discussed as potentially having difficulty translating to detecting virus particles, which are typically orders of magnitude smaller in diameter. Table 4 shows a breakdown summary of critical attributes of the methods discussed in these finalized papers.

A key difference seen with publications on biosensors for airborne detection (Table 4) versus the main trends seen for general SARS-CoV-2 detection (Table 3) is the use of combined sampling and collection methods. In Table 3, we noticed that most papers used an impactor, typically a commercially purchased one, combined with nucleic acid detection. However, in Table 4, we can see that most air sampling methods combined an air pump with an additional enrichment method, such as electrostatic precipitation or microfluidics.

Viability is a crucial issue, especially when it comes to bacterial aerosol detection. To culture collected microorganisms, a live sample is required. High flow samplers put bioaerosols through high shear forces, which can then damage the cell. Liquid samplers also put samples under high shear forces; however, there appears to be less damage than non-liquid samplers such as impactors and pumps. In addition, many viruses are not culturable, and nucleic acid detection techniques or immunoassay techniques do not require live samples, so these concerns are not as prevalent.

Airborne viruses are difficult to detect because of their low concentration. Optimized sampling and collection methods are used to improve the collection efficiency of airborne particles. While the LOD of nucleic acid amplification (e.g., RT-qPCR) has generally been considered much lower than antibody-antigen methods (e.g., ELISA and other immunoassays), the LOD's shown in Table 4 do not show substantial differences between these two methods. Lowest LOD's were 10 copies/µL (= 10^4 copies/mL) and 138 pg/mL for antibody-antigen methods, and 30 copies and 6 PFU/mL for PCR methods[56,57,66,72-83]. Medium-to-high LOD's were around 10^6 PFU/mL for both antibody-antigen and PCR methods[56,57,66,72-83]. LOD's are more affected by the collection efficiency, which can be varied by the device's flow volume, presence or absence of applied charge, and enrichment steps.

Table 4. Overview of articles demonstrating biosensors for the bioaerosol detection.

Ref.	Collection method	Air flow rate, L/min	Collection time, min	Enrichment Method	Detection method	Detection time, hr	Target analyte	LOD	Key biosensor attributes
Bacteria detection using biosensors									
Chen & Yao[72]	Impactor	1200	<10	N/A	Culturing, gene sequencing	24-48	Bacteria (many species)	N/A	Portable
Kim et al.[73]	Electrostatic, ATH enrichment	4-10	2	HTH, magnetic particles	Real time-qPCR	1-4	*S. aureus, B. cereus, E. coli*	4.75×10^7 CFU/m^3 4.63×10^5 CFU/m^3 1.5×10^6 CFU/m^3	
Jiang et al.[74], Jing et al.[75]	Pump + microfluidic	0.001-0.012[75]	<60	Microfluidic chip	LAMP + fluorescence	1.5	*S. aureus*	24 CFU/mL	No DNA purification
Choi et al.[76]	Inertial microfluidics with two-phase continuous flow	0.6	10	N/A	Culturing, particle counting	N/A	*S. epidermidis*	918 CFU/mL	High collection efficiency
Choi et al.[77]	Pump + SERS optofluidic platform	0.2-1.2	1	N/A	AgNP + SERS spectra	Real-time	*S. epidermidis*	100 CFU/mL	AgNP in liquid collection
Virus detection (not necessarily coronavirus) using biosensors									
Ladhani et al.[78]	Pumps + electrostatic precipitation with liquid collector	6.7	15	N/A	RT-qPCR	1-4	Infl. A/H1N1, H3N2	303-3721 RNA copies	
Hong et al.[79]	Electrostatic	1.2	10	N/A	qPCR, plaque assay	1-4	MS2 & T3 phage	N/A	Portable; High virus

|									viability
Lee et al.[80]	LFA	75-100	<30	Concentration step on sampling pad	Immuno-fluorescence assay	0.33	MS2 phage Avian infl.	10^6 PFU/mL $10^{3.5}$ EID_{50}/m^3	
Lee et al.[81]	3D photonic crystal	5	N/A	N/A	Fluorescent probe, FRET	0.67	Infl. A/H1N1	138 pg/mL	Portable; Low-cost
Usachev et al.[82]	Pump + collection liquid (bubbler)	4	10	N/A	SPR spectra, immunoassay	1 min	MS2 phage Infl. A/ H1N1	6×10^6 PFU/mL 7×10^5 PFU/mL	
Agranovski & Usachev[83]	Pump + collection into liquid	N/A	0.17-10	N/A	PCR	<0.67	T4 phage	10^6 PFU/mL	Handheld
Piri et al.[57]	ATH EP sampler	8	N/A	N/A	ELISA PCR	N/A	Infl. A/H1N1 HCoV-229E	N/A 1.5×10^7 PFU/mL	Reduced damage to virus
Kim et al.[56]	Electrostatic, ATH enrichment	4-10	N/A	HTH, magnetic particles	RT-qPCR	1-4	HCoV-229E Infl. A/H1N1	6 PFU/mL 55 PFU/m^3	
Xiong et al.[66]	Air sampler + microfluidic fluorescence system	50-250	30	N/A	LAMP	0.25	SARS-CoV-2	10 copies/μL	Portable; Multiple sample processing

Note. Infl. = influenza. Phage = bacteriophage.

Sampling Methods

Flow volume is a common characteristic referenced in bioaerosol sampling. In general, higher flow samplers are desirable because they bring a larger volume of air into contact with the collection device and subsequently improve chances of detection[72]. However, high flow rates may also affect bioaerosol vitality. The HighBioTrap device used by Chen & Yao[72] (Figure 5C) achieves a very high flow volume of 1200 L/min. Tested with polystyrene particles down to 0.3 μm (larger than SARS-CoV-2 with 0.1 μm diameter), it reached a collection efficiency of 10%. The HighBioTrap was also tested on bacteria and achieved a 20% collection efficiency, with only 3-5 minutes of air sampling time[72]. In contrast to the HighBioTrap device, a lower flow volume can be used to prioritize maintaining virus viability. Gentle sampling with a very low flow rate was achieved using electrostatic bioaerosol collection devices[57,79,84]. Hong et al. developed a personal electrostatic particle concentrator with very high collection efficiencies of 99.3-99.8% for 0.05-2 μm diameter polystyrene particles using a very low flow rate of 1.2 L/min[79]. The high collection efficiency with gentle sampling was achieved due to the enhanced electric field strength used (Figure 5B)[79]. This is desirable so that the collected bioaerosols do not lose viability during sampling, which is important if the goal is to estimate actual viable airborne concentrations of particles and is therefore relevant to consider. The efficiency was the highest for smaller particles, promising for potential translation to virus detection. This technique not only achieved high collection efficiency but also had a recovery rate > 900X larger than in the commercial SKC BioSampler, which uses a 12.5 L/min flow rate[79].

Viability does not have to be a primary focus for successful airborne virus detection, as virus fragments can still be detected, but the very high collection efficiency is a helpful factor for virus detection considering the low initial airborne concentration.

The combined use of microfluidics and electrostatic precipitation (ESP)-based sampling offer comparable collection efficiencies to commercial biosampling impingers[84]. The proposed point-of-care (POC) device (Figure 5A) was a conceptual development for directly collecting droplets using a microfluidic platform. The capture of the droplets uses corona discharge, similar to the gentle sampler by Hong et al. discussed previously[79], allowing for different distances and currents to optimize the parameters. This dynamic prototype showed a maximum collection efficiency of 21% from small 300 μL samples. Compared to the consistent 19% rate of a commercial impinger from 4 mL samples, these appear to be competitive results. Without ESP the collection efficiency was less than 1%, so this suggests that microfluidic devices may especially require additional help for achieving reasonable collection efficiencies. Pardon et al. claims this is suitable for POC diagnostics for human exhaled breath aerosols, following further research into effects of patient breathing patterns and power supply, among others[84]. Although this device was not tested directly on aerosolized viruses, its research on the effectiveness of ESP and potential for microfluidic collection is interesting and applicable to further investigation.

Figure 5 summarizes the electrostatic bioaerosol collecting method.
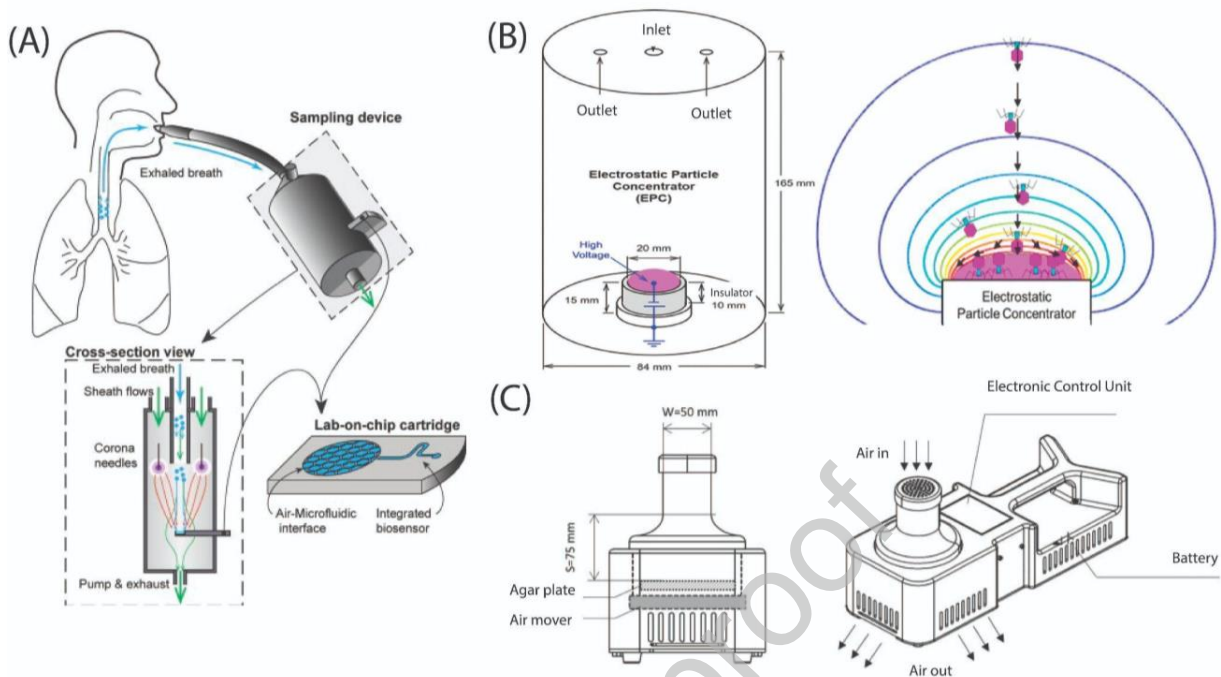
Figure 5. Electrostatic samplers for bioaerosol detection. A) Schematic of electrostatic precipitator (ESP) as a sampling device. Droplets are captured onto a microfluidic air-to-liquid interface for later analysis. Reprinted from Pardon et al. ref. 84 with permission, (c) 2015 Elsevier. B) Schematic of the electrostatic particle concentrator (EPC) for gentle sampling of submicrometer airborne virus particles. Reprinted from Hong et al. ref. 79 with permission, (c) 2016 Elsevier. C) 3D sketch of the HighBioTrap, a battery-powered high-flow sampler. Reprinted from Chen & Yao ref. 72 with permission, (c) 2018 Elsevier.

While Ladhani et al. used electrostatic precipitation to improve the collection efficiency of their microfluidic device[78], some other authors have utilized enrichment methods. Enrichment steps help to lower the limit of detection (LOD) by increasing particle concentration by collecting aerosols into a liquid, causing a volume reduction and increased sample concentration[51,52,66]. This is similar to the concentration step used to artificially increase the norovirus captured onto a microfluidic chip in this liquid-based immunofluorescence assay[85,86]. Enrichment steps are especially advantageous for respiratory virus biosensors, due to their low airborne concentration. Aerosol-to-hydrosol (ATH) enrichment is the most effective for higher flow volumes[57,73] ATH is both air sampling and enrichment step, capable of achieving up to 80,000x enrichment capacity[73] Particle collection efficiency increases with particle charge, and therefore a negative voltage is often applied[20]. The simultaneous liquid and air collection process is clearly very efficient at reaching high collection percentages and should be investigated for the application of airborne virus detection. Enrichment capacity (EC) is used to evaluate the extent to which particle concentration can be increased.

Although ATH enrichment is the most typical, hydrosol-to-hydrosol (HTH) enrichment can also be used to incorporate biomarkers for improved detection. Following ATH sampling

and enrichment, Kim et al. used concanavalin A (ConA)-coated magnetic particles (CMPs) placed in the fluidic channel to achieve an additional 14.9 enrichment capacity, totalling 1.192×10^5 enrichment capacity in just 1 minute. This was tested and shown to be over 1000 times better than the commercial SKC BioSampler[73]. This method was also tested on airborne HCoV-229E and influenza A/2021[56], which will be discussed in the following paragraph.

Microfluidic platforms offer opportunities for rapid, sensitive, and *in situ* detection of airborne respiratory viruses. The combination of enrichment and microfluidic platforms could maintain low LODs while maintaining rapid and portable capabilities. Jiang et al. investigated such a combination (Figure 6B) with the use of microfluidics for enrichment and detection of airborne bacteria using high-throughput LAMP (loop-mediated isothermal amplification; a nucleic acid amplification method that runs on a single temperature) analysis with a LOD down to 24 cells per reaction and binary detection from the naked eye[74]. Another exploration of microfluidics for enrichment was done by Choi et al. (Figure 6A) who used inertial microfluidics for enriched and continuous aerosol sampling[76]. Similar to ATH enrichment methods, the extent of particle collection was increased by sampling air and collecting liquid simultaneously and continuously. Polystyrene particles of 1 μm could be collected with up to 98% efficiency. This technique was shown successful for bacteria and polystyrene particles ranging from 0.6-2.1 μm[76].

These microfluidic enrichment devices are summarized in Figure 6.
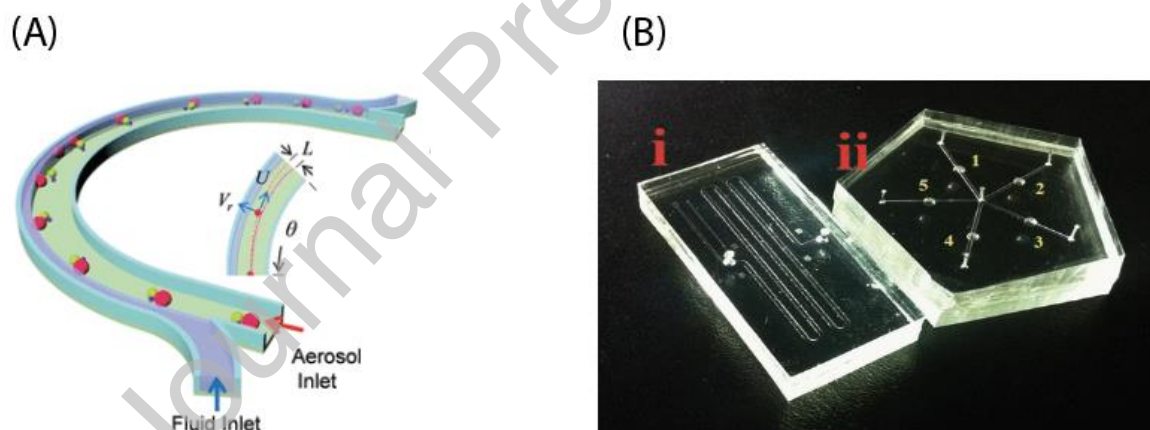
Figure 6. Microfluidic enrichment devices for detecting bacteria or polystyrene (surrogate) particles from droplets and aerosols. A) Schematic of the MicroSampler, an inertial microfluidic technique for continuous aerosol sampling; Reprinted from Choi et al. ref. 76 with permission, (C) 2017 American Chemical Society. B) Photograph of the airborne bacterial capture and enrichment (i) and high-throughput LAMP chip (ii); Reprinted from Jiang et al. ref. 74 with permission, (C) 2016 American Chemical Society.

Ladhani et al. continued the research on POC applications for detection of airborne influenza A/H1N1 using electrostatic precipitation (ESP)-based bioaerosol samplers, with RT-qPCR for detection and analysis[78]. Sample volume used was 150 μL, improving upon other

liquid-based aerosol biosamplers that are typically in the mL range[78]. The collection efficiency demonstrated with this technique was > 10% but a second extraction protocol further improved collection efficiency up to 47%. Collection efficiencies were compared to gelatin filters, which are a good standard and get close to 100% efficiencies[78].

Detection Methods

Many airborne microorganism detections are done by nucleic acid detection, most commonly PCR. The methods and devices summarized in Figures 5 and 6 are focused on bacteria and polystyrene (surrogate) particles, and their detections are still conducted in a conventional manner, i.e., culturing and RT-PCR. While the use of RT-qPCR is neither rapid nor novel, it is advantageous because it can be used with clinical samples. Culturing is not an option for many virus types. Nucleic acid amplification requires more time than some alternative methods, such as optical measurement[77], however produces very low false-positives[87].

After collecting influenza A/H1N1 particles using the ESP sampler, Ladhani et al. used RT-qPCR with additional extraction protocols to improve their LOD of 3721 RNA copies to 303 (n=1 data point) RNA copies[78]. Ladhani et al. thoroughly tested different extraction protocols comparing the effectiveness of using universal transport medium (UTM) rinsing and filter wiping[78].

Laboratory-based PCR can also be implemented within a biosensor using a portable and relatively rapid PCR device. Multiplex PCR using a single PCR tube and single fluorescent dye was investigated using a mini-PCR device, with intersample variability remaining less than 10%[83]. This device showed high efficiencies at 10 minutes, 1 minute, and 10 seconds of bioaerosol sampling time but only 50% collection at 5 seconds sampling time. The mini-PCR device uses a laser diode for precise excitation, eliminating the need to use optical filters, and performs rapid heating and cooling using a thermistor and air fan[83]. It appears like the portable PCR device could be combined with any other sampling system optimized for the target virus of choice.

Optical measurement methods allow for immediate analysis of collected particles, ideal for point-of-care and rapid detection of airborne virus particles. Similar to the inertial microfluidic platform with simultaneous liquid and air collection[76], Choi et al. presented a modified technique with the addition of surface-enhanced Raman spectroscopy (SERS) for detection of the collected airborne bacteria in continuous real-time manner[77]. Spectra were collected over 60s, with bacterial concentration increasing the most after 15 minutes of collection time. Collection efficiency was 99.6% for 1 μm particles, and the LOD was approximately 100 CFU/mL for total bacterial aerosol concentration[77].

Combined Sampling and Detection

Since microfluidic devices have popularly been demonstrated for biosensor applications, integrated microfluidic devices have been developed that can conduct both sampling and detection, although the number of such work is still small.

Figure 7 summarizes the integrated sampling and detection devices.
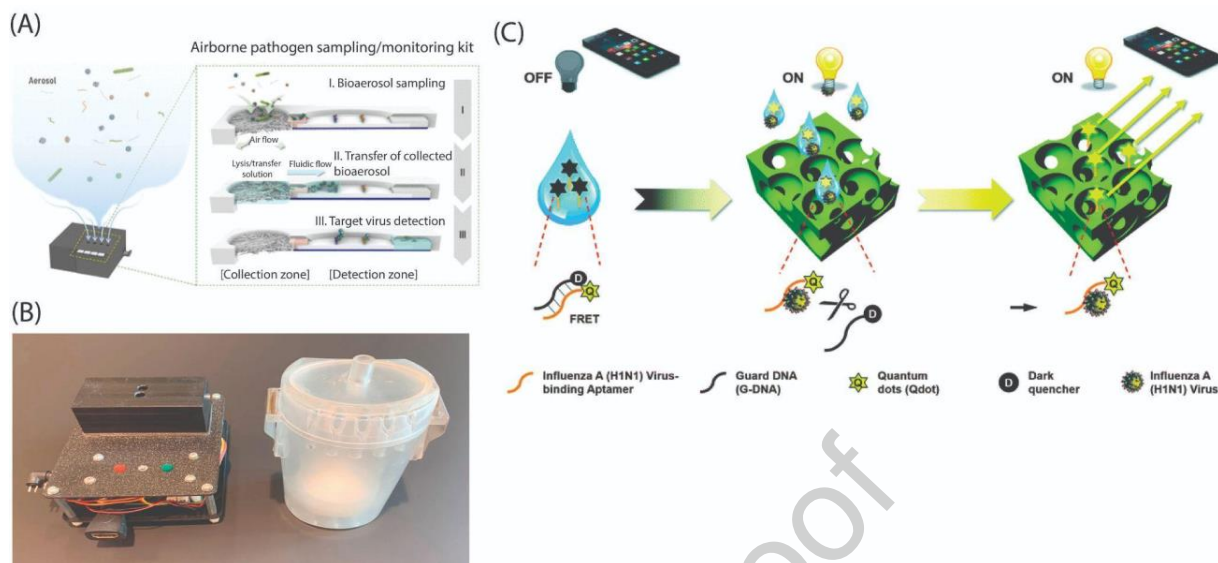
Figure 7. Integrated sampling and detection microfluidic devices. A) 3D schematic of integrated sampling and monitoring platform. Air samples are collected and processed on the lateral flow assay using fluid flow and lanthanide-doped upconversion nanoparticles (UCNPs); Reprinted from Lee et al. ref. 80 with permission, (c) 2020 American Chemical Society. B) Miniature PCR machine that is portable and lightweight (left) and accompanying personal bio-aerosol sampler filled with collecting liquid (right); Reprinted from Agranovski & Usachev ref. 83 with permission, (c) 2020 Elsevier. C) Quenched Qdot-aptamer conjugates are loaded into aerosol spray and photonics crystal is in 'off' stage (left). Aerosolized virus is captured on the photonics crystal triggering 'on' state (middle). Signal is detected and measured using smartphone (right); Reprinted from Lee et al. ref. 81 with CC BY license, (c) 2018 Lee et al.

Lee et al. proposed an integrated sampling and monitoring platform for rapid detection of influenza A/H1N1 and bacteriophage MS2 (a virus of roughly 28 nm), using a paper-based lateral flow immunochromatographic assay (LFA) for both collection and detection of aerosolized virus[80]. This approach is highly novel for airborne virus detection as no other papers discussed the use of LFAs for rapid aerosol detection. Lee et al. used glass fiber pads as the aerosol sampling pad, which is directly connected to the LFA test strip[80]. After collection, a loading buffer is injected through the glass fiber to lyse any deposited pathogens. The target virus was then captured in the detection zone of the LFA by lanthanide-doped upconversion nanoparticles (UCNPs), which emit near infrared (NIR) wavelengths so that the captured airborne pathogens can be immediately detected using NIR-to-NIR nanoprobes for target pathogen detection. The integrated lateral flow assay was placed in a small chamber that simulated an indoor room environment, and the air sampler was operated for 15 minutes at 100 L/min. Lee et al. suggest that the collection efficiency can be further improved by including eluting steps such as centrifugation or vortexing to improve virus transfer from the glass fiber sample pad[80].

Nanoparticles are particularly useful for specific detection of target pathogens. Lee et al. used quantum dot (Qdot)-aptamer beacons and light guiding in a 3D photonic crystal for quantification of airborne influenza A/H1N1. POC detection of H1N1 using crystal-based sensors, specifically a quartz crystal microbalance (QCM)-based sensor can provide a unique alternative to bulky samplers[81]. This technique measures changes in resonance frequency to quantify deposition of airborne viruses on the sensor surface. The fluorescent signal, enhanced using dark quencher-labeled guard DNA (G-DNA) from the Qdot-aptamer beacons, was used as an 'off-on' binary detection method and quantitative tool for determining concentrations down to 138 pg/mL. The additional method discussed uses a smartphone camera setup, costing $20 USD, which reaches an LOD of 70 ng/mL when testing serum[81]. While this technique is exciting, it does not thoroughly experiment with aerosolized viruses, and the LODs may not represent real state air detection.

Surface plasmon resonance (SPR) offers rapid and precise detection of target viruses. A combined multiplex SPR and a bubbler type bioaerosol sampler for rapidly detecting multiple airborne pathogens showed low LOD and high specificity in both singleplex and multiplex sensors[82]. Bacteriophage MS2 was tested on a singleplex sensor with only anti-MS2 antibodies and a multiplex sensor with anti-influenza A antibodies as well. For bacteriophage MS2, LOD was found to be 6×10^6 PFU/mL for both single and multiplex sensors, and for influenza A the LOD was 7×10^5 PFU/mL[82].

Recent Biosensor Advances for Airborne Coronaviruses

The final few papers we will now review specifically focus on detection of airborne coronaviruses[56,57,66,71,88], and one that does not focus on airborne detection but could potentially be applied to bioaerosols[89]. Two review papers present summaries of sensor technologies for bioaerosol detection, including examples for SARS-CoV detection. Su et al.'s review highlights that SARS-CoV-2 RNA was found from air samples using impinger and filter collection plus RT-PCR detection[88], however does not reference any biosensors for virus detection published later than 2015. Rahmani et al.'s review focuses specifically on detection of airborne coronavirus by looking at benefits of different sampling characteristics, such as sampling time and flow rate, as well as culturing and detection, including RT-PCR and reverse transcription loop mediated isothermal amplification (RT-LAMP) techniques[71].

There are two research papers that used HCoV-229E. Piri et al. used ESP with ascorbic acid (AA) dissolved in phosphate-buffered saline (PBS) to sample bioaerosols as hydrosols with increased survivability (Figure 8A)[57]. Additional steps are needed in ESP in order to reduce damage to the viral species, in particular HCoV-229E which showed greater impairment due to corona discharge exposure than influenza A/H1N1[57]. ESP produces corona discharge which ionizes the air and generates reactive oxygen species (ROS) and reactive nitrogen species (RNS). However, using the PBS+AA method reduces this damage to viral RNA, viral protein, and general viral yield by 95%, 45%, and 60% respectively. The PBS+AA treatment is the main

novel aspect of this paper, the air sampler used was developed previously, and the virus was collected by exposing the virus hydrosols using a peristaltic pump. Samples were tested using conventional laboratory analyses: virus culturing, enzyme linked immunosorbent assay (ELISA), and RT-PCR[57].

Simultaneous ATH and HTH enrichment was also used for human coronavirus 229E with enrichment capacities up to 67,000 virus particles. This improved the LOD to detect the HCoV-229E with the average RT-qPCR threshold cycle values (Ct) of 33.8[56]. Kim et al. used an electrostatic air sampler (Figure 8B) to capture aerosolized HCoV-229E, influenza A/H1N1 and H3N2, as well as ATH and HTH fluidic enrichment system[56]. Detection was done in a conventional RT-PCR.

Finally, there are two research papers that used SARS-CoV-2. Xiong et al. presented an exciting application of microfluidics towards SARS-CoV-2 detection. They successfully performed tests of 115 samples in a non-laboratory setting. This method used a combined sampling and monitoring platform with a rotating polycarbonate microfluidic fluorescence chip-integrated aerosol sampler and monitor[66]. This system was small volume and highly sensitive, achieving the LOD of 10 SARS-CoV-2 copies/μL with CV < 5%. The microfluidic chip has zones for sample lysing, DNA separation, and amplification. Four chambers are available, each requiring 5 μL. Xiong et al. targeted the SARS-CoV-2 O gene, N gene, internal standard gene, and a blank control. This system is quicker than some alternatives, however requiring 75 minutes total turnaround time for one device, still able to achieve 96 samples per device per day[66]. This paper is one of the only ones we found that tested clinical samples.

There is one additional paper by Qiu et al. that detected SARS-CoV-2, but not from aerosol samples. A dual-functional plasmonic biosensor using plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction was shown to detect SARS-CoV-2 sequences down to 0.22 pM, corresponding to approximately 113 copies/μL, from a multigene mixture[89]. This system (Figure 8D) is not portable and was not tested on airborne samples; however, the high sensitivity and specificity could be further tested for detection of SARS-CoV-2 from airborne multiplex samples.

Figure 8 summarizes the biosensor detection of coronaviruses (HCoV-229E and SARS-CoV-2).
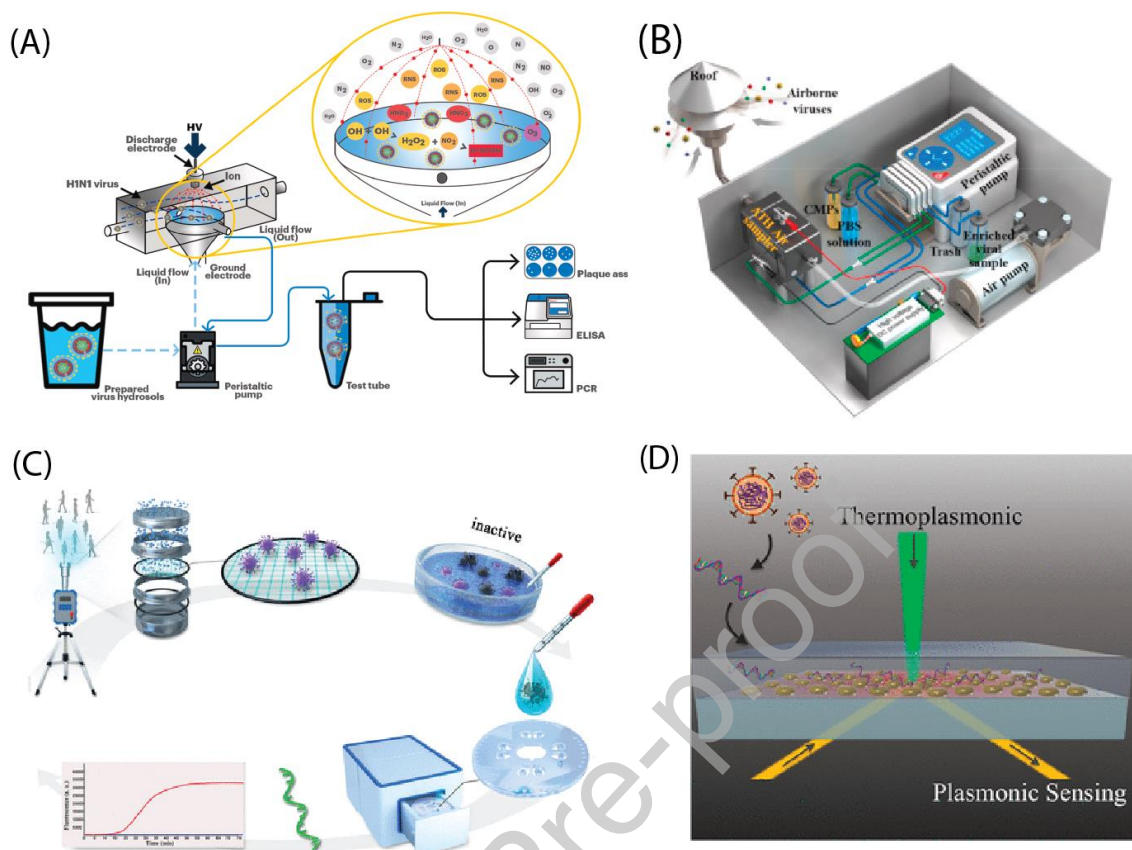
Figure 8. Biosensors for detecting airborne coronavirus (HCoV-229E; not SARS-CoV-2). A) Schematic of electrostatic aerosol-to-hydrosol sampler; Reprinted from Piri et al. ref. 57 with permission, (c) 2021 Elsevier. B) 3D schematic (left) and photograph (right) of the integrated system for HTH or ATH air sampling plus additional enrichment of airborne virus particles; Reprinted from Kim et al. ref. 56 with permission, (c) 2020 Elsevier. C) Schematic diagram of the integrated sampling/monitoring microfluidic platform; Reprinted from Xiong et al. ref. 66 with permission, (c) 2021 American Chemical Society. D) Schematic setup of the dual-functional PPT enhanced LSPR biosensing system; Reprinted from ref. 89 with CC BY license, (c) 2020 Qiu et al.

Concluding Remarks and a Proposal

There is a clear need for continued research in the realm of biosensors for airborne virus detection. The development of rapid and point-of-care (POC) devices can increase the ability for hospitals, public spaces, and private residences to detect and prevent virus contamination. To successfully meet the goal of an environmental biosensor for detection of airborne respiratory viruses like SARS-CoV-2, we propose an automated, easily deployable system. The proposed system will ideally be (1) handheld for ease of use, (2) relatively inexpensive, (3) easily reproducible, (4) sensitive, (5) compatible with smartphone platforms (e.g., iPhone and Android), (6) adaptable to a range of respiratory viral targets, and (7) provide rapid feedback to users.

Based on the papers reviewed in this publication, as well as the personal field of research of the authors, our recommendation is the development of a paper-based microfluidic system for sampling and collection of airborne SARS-CoV-2. The microfluidic chip (Figure 9) could be stored in an electrostatic sampling device, similar to the electrostatic precipitator seen in Figure 6B. Depending on the priority of the device, either a high flow sampling rate or a gentle and slower sampling rate could be used to increase capturing efficiency or virus viability respectively. This device would be portable and potentially highly efficient. Models on air flow in indoor environments can help gauge ideal positioning of these devices, possibly in areas of increased airborne virus concentration such as near hospital beds or in restrooms. After collection of airborne particles onto the microfluidic chip, either an immunofluorescence assay or a nucleic acid amplification technique can be used to identify virus presence and concentration.
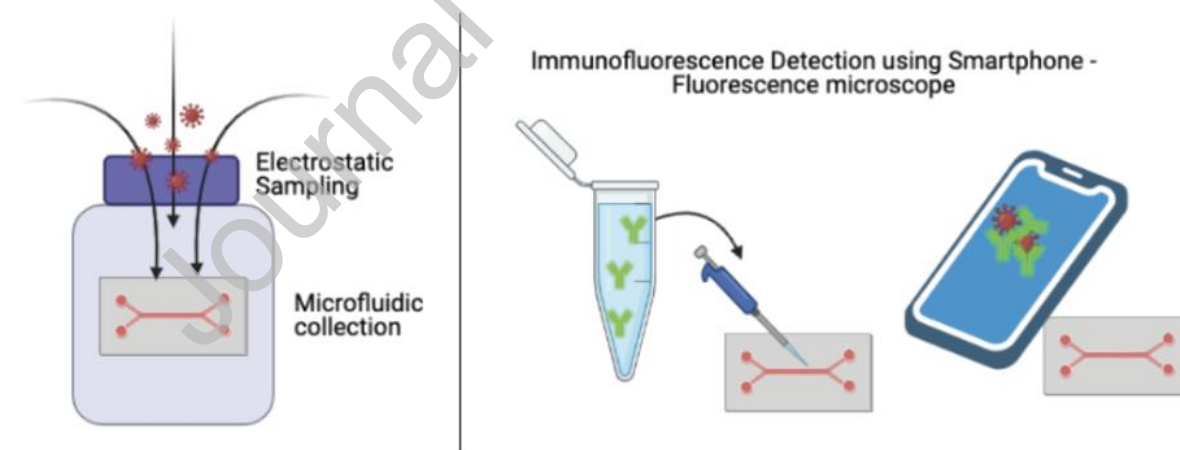
Figure 9. Schematic for a SARS-CoV-2 detection system using electrostatic sampling and collection onto a microfluidic platform, followed by immunofluorescence detection using anti-SARS-CoV-2 antibodies conjugated to fluorescent particles. Levels of immunoagglutination are quantified using smartphone-based fluorescence microscope for diagnosis of coronavirus presence or not. Schematic was made using BioRender.com.

If using immunofluorescence detection, then a smartphone-fluorescence microscope could be used to portably detect immunoagglutination between positive virus samples and target antigen specific antibody-conjugated fluorescent particles. This device will consist of three central components: (1) the paper-based microfluidic chip with wax-printed channels, (2) an imaging attachment, and (3) a smartphone with analysis and user interface software. The microfluidic chip will be designed such that immobilized antibodies are preloaded to target respiratory virus antigens. Conjugation of fluorescent particles to the antibodies will allow for sensitive imaging and quantification of captured virus, as we have previously demonstrated for norovirus detection[85]. Using the immunofluorescence assay on a lateral flow microfluidic chip has achieved LODs down to single virus copy level (corresponding to 1 copy/mL or 1 fg/uL norovirus virions), as it can detect antigens and virus fragments. It could be expected to achieve similar results with coronavirus. Imaging will be performed using a microscope attachment, an excitation LED, and proper excitation/emission filters. Smartphone software will control the capture of images and automatically analyze the samples to provide results within minutes.

An alternative to immunofluorescence assays is to take samples from the microfluidic chip and perform nucleic acid amplification methods, such as RT-qPCR. This nucleic acid detection could further lower the LOD and increase specificity due to the high accuracy of nucleic acid techniques. Although amplification methods would take longer than using immunofluorescence, using the microfluidic chip as the platform could still permit portable virus collection.

These proposed systems allow for microfluidic chips to be placed in a plethora of regions for comprehensive coverage of airborne virus collection in any desired setting. Optimization of the sampling flow rate and detection technique will emphasize either rapid detection or highly accurate quantification. Continued characterization of airborne viruses, such as SARS-CoV-2, will help improve the ability to construct devices optimized for the target pathogen, as well as detection methods which are highly specific.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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